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De Novo Biosynthesis of Volatiles Induced by Insect Herbivory in Cotton Plants

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In response to insect feeding on the leaves, cotton (*Gossypium hirsutum* L.) plants release elevated levels of volatiles, which can serve as a chemical signal that attracts natural enemies of the herbivore to the damaged plant. Pulse-labeling experiments with [^{13}C]CO_2 demonstrated that many of the volatiles released, including the acyclic terpenes (*E,E*)-α-farnesene, (*E*)-β-farnesene, (*E*)-β-ocimene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, as well as the shikimate pathway product indole, are biosynthesized de novo following insect damage. However, other volatile constituents, including several cyclic terpenes, butyrates, and green leaf volatiles of the lipoxygenase pathway are released from storage or synthesized from stored intermediates. Analysis of volatiles from artificially damaged plants, with and without beet armyworm (*Spodoptera exigua* Hübner) oral secretions exogenously applied to the leaves, as well as volatiles from beet armyworm-damaged and -undamaged control plants, demonstrated that the application of caterpillar oral secretions increased both the production and release of several volatiles that are synthesized de novo in response to insect feeding. These results establish that the plant plays an active and dynamic role in mediating the interaction between herbivores and natural enemies of herbivores.

Volatile plant compounds released in response to insect feeding can serve as chemical signals for herbivore natural enemies. These volatile cues, which guide host-seeking parasites or predators to insect-damaged plants, operate in several agricultural species, including corn (*Zea mays* L.) (Turlings et al., 1990), lima bean (*Phaseolus lunatus* L.) (Dicke et al., 1990; Takabayashi and Dicke, 1996), cotton (*Gossypium hirsutum* L.) (McCall et al., 1993, 1994; Loughrin et al., 1995), and brussels sprouts (*Brassica oleracea gemmifera*) (Mattiacci et al., 1994). Once a parasitoid wasp has located a host larva, she injects her eggs into the herbivore, which shortens the feeding life of the caterpillar and terminates its reproductive cycle while propagating the wasp's own species (Tumlinson et al., 1993; Turlings et al., 1993b). Although the connection between damage-released plant volatiles and the attraction of herbivore parasites and predators has been demonstrated in several cases, the sequence of plant biochemical reactions that occur in response to herbivore feeding that triggers volatile release is not well understood (Paré and Tumlinson, 1996). This laboratory has recently shown that BAW-damaged cotton releases some volatiles at a relatively steady rate that appears to correlate with caterpillar feeding, whereas other compounds, mostly acyclic terpenes and indole, are released in a diurnal cycle, with much higher levels of volatiles released during the day than at night (Loughrin et al., 1994). The delay of almost 24 h between the start of BAW feeding and the release of certain volatiles suggests that a series of inducible biochemical reactions is required for these compounds to be released.

One proposed mechanism for this delay is that plants store certain volatile compounds as glycosides, which are cleaved by glycosidases present in insect saliva, resulting in volatile release (Boland et al., 1992). In fact, β-glucosidase activity has been measured in the regurgitant of *Pieris brassicae*, a herbivore that feeds on cabbage, and leaves treated with a commercial β-glucosidase released a volatile blend similar to that of leaves treated with *P. brassicae* regurgitant (Mattiacci et al., 1995).

Although a second research group has also reported on glucosidases as elicitors of plant volatiles (Boland et al., 1992), some of their studies of the biosynthesis of homoterpenes, a group of compounds formed by a series of degradative steps from either a sesquiterpene or diterpene precursor (Donath and Boland, 1994), suggest that these compounds are synthesized de novo in response to insect feeding rather than just being released by hydrolysis of the corresponding glycosides. This includes a report of the absence of stored glycosides that could act as homoterpene precursors in lima bean (Hopke et al., 1994) and experiments showing that the release of homoterpenes is almost exclusively from applied precursor substrates for a variety of plant species in the absence of insect damage (Gäbler et al., 1991). If homoterpenes were routinely sequestered by undamaged plants, the application of the homoterpene precursors nerolidol or geranylinalool to undamaged leaves would not be expected to lead to homoterpene release. The homoterpene pathway may therefore be blocked upstream of the substrates exogenously added so that homoterpene synthesis does not occur unless plants are wounded and the full pathway is activated.

In a brief communication we reported that BAW feeding on cotton leaves triggered de novo biosynthesis of several volatile compounds (Paré and Tumlinson, 1997). Here we report the synthesis from three separate biochemical routes

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Abbreviations: ANOVA, analysis of variance; BAW, beet armyworm; *m/z*, mass to charge ratio.

(Fig. 1) of a blend of cotton volatiles released in response to insect feeding. We investigated the timing of synthesis and metabolism of herbivore-induced volatiles by conducting pulse-chase labeling experiments with $^{13}CO_2$. To examine the role of caterpillar feeding on the release of these airborne compounds, the volatile profile from caterpillar-damaged plants was compared with the compounds released from plants after repeated mechanical damage with or without exogenously applied oral secretions from insects.

MATERIALS AND METHODS

Plant Growth and Insect Rearing

Cotton (*Gossypium hirsutum* L., var Deltapine Acala 90) plants grown from seeds were maintained in an insect-free greenhouse. Room temperature was maintained at 29 ± 4°C with a RH minimum of 40 ± 5% in the late afternoon and a maximum of 95 ± 5% in the early morning. The greenhouse is equipped with 400-W high-pressure sodium lamps to supplement natural light with a 16-h light/8-h dark photoperiod from November through February. Plants were grown in 16-cm-diameter pots using Metromix 300 potting soil (Scotts-Sierra Horticulture, Marysville, OH). Six-week-old plants that were 25 to 30 cm tall and had not set flower buds were used in all of the labeling studies. BAWs (*Spodoptera exigua* Hübner) were reared on an artificial diet in this laboratory by the method of King and Leppla (1984). Fourth-instar caterpillars were starved for 7 h prior to being placed on plants.

Plant Wounding

Five larvae were placed on a plant at the start of the experiment and were allowed to feed continuously while plants were in the volatile collection apparatus (see "Collection and Identification of Volatile Compounds"). The hole at the bottom of the chamber around the plant stem was loosely plugged with cotton, which prevented larval escape. Plants were mechanically damaged by puncturing the lower leaves with a plastic "derma-pik" (40-mm length × 2-mm diameter), which has six tines arranged in a circle around the perimeter of the tip (Greer Laboratories, Lenoir, NC). This end was dipped into BAW oral secretion or buffer and the droplet of the solution was applied to the leaf at the site of wounding. Forty microliters of an oral secretion or buffer (50 mM sodium phosphate/citric acid, pH 8.0) was measured and applied via the derma-pik to one of the leaves every 2 h during a 51-h period. Caterpillars were removed at the end of the artificial damage treatment and leaves were photocopied the following morning. Total leaf area and insect-damaged portions were measured by scanning a photocopy of the leaves (Sigma Scan, version 3.0, Jandel Scientific, Sausalito, CA).

Oral secretion was collected from BAW larvae that had fed on freshly cut corn (*Zea mays* L.) leaves for 1 or 2 d. Regurgitation was induced by holding the caterpillar with forceps and gently pinching the head region with a second pair. The oral secretion was collected via a 100-μL pipet inserted into a vial, with the apparatus under a low vacuum (Turlings et al., 1993a), and stored at −5°C until used.

Collection and Identification of Volatile Compounds

The aerial portion of the plants was contained within a 34-cm-tall × 6-cm-diameter glass cylinder, which had a split plate with a hole in the center at the base that closed loosely around the stem of the plant like a guillotine. Air entered the top of the glass sleeve through five layers of an activated charcoal-infused fabric and passed over the plant at a rate of 1 L min^{-1} (Manukian and Heath, 1993; Heath and Manukian, 1992). Plant volatiles were collected at 3-h intervals by pulling one-half of the air (0.5 L min^{-1}) that passed over the plant through Super-Q adsorbent traps (Alltech, Deerfield, IL) located around the base of the collection chamber; the remainder of the air was vented out the bottom of the system. Volatiles were collected within the greenhouse. Temperature and humidity were recorded at 2-min intervals using a programmable data-logger with built-in sensors (Analytical Research Systems, Gainesville, FL). Within the glass chambers the temperature decreased to 21°C (minimum) at night and went up to 38°C (maximum) in the afternoon; the RH ranged from 85 to 100%.

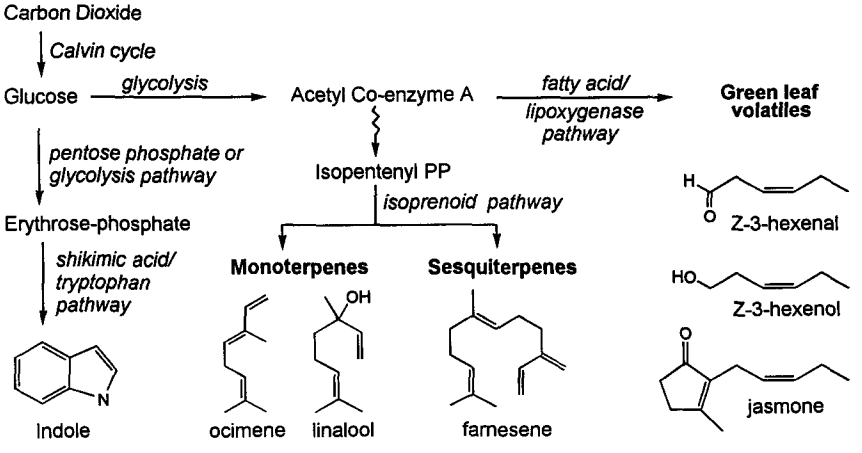

Figure 1. The biosynthetic routes leading to three classes of volatiles (indole, terpenoids, and green leaf volatiles) that are released from cotton plants with BAW feeding on the leaves. The squiggly arrow pointed toward isopentenyl pyrophosphate represents the current uncertainty of this biosynthetic route (Eisenreich et al., 1996).

Compounds were eluted from the adsorbent traps with 150 μL of dichloromethane; 400 ng each of *n*-octane and nonyl acetate were added as internal standards and 1-μL aliquots were analyzed by capillary GC on a 50-m × 0.25-mm (i.d.) fused silica column with a 0.25-μm-thick bonded methyl silicone stationary phase (Quadrex, New Haven, CT). Injections were made in the splitless mode for 30 s, and the gas chromatograph was operated under the following conditions: injector 220°C, detector 220°C, column oven 60°C for 2 min, then programmed at 4°C min^{-1} to 180°C, and He carrier gas linear flow velocity 19 cm s^{-1}. Selected samples were also analyzed by GC-MS on a (ion trap) mass spectrometer (MAT ITS40, Finnigan, Austin, TX) interfaced to a gas chromatograph (model 3400, Varian, Sunnyvale, CA) and operated in the electron impact mode. Injections were made via a septum-equipped programmable injector held at 40°C for 0.25 min, then programmed at 170°C min^{-1} to 270°C onto a 30-m × 0.25-mm (i.d.) fused silica column with 0.25-μm-thick bonded 5% phenyl methyl silicone (DB-5MS; J&W Scientific, Folsom, CA) held at 40°C for 5 min, and then programmed at 5°C min^{-1} to 260°C; He carrier gas linear flow velocity was 19 cm s^{-1}. The source temperature was adjusted to 120 ± 20°C to optimize the molecular ion abundance. The components of the plant volatile emission were identified by comparison of GC retention times with those of authentic standards on both capillary columns and by comparison of mass spectra with spectra of an Environmental Protection Agency/National Institutes of Health database.

In Vivo Labeling

Synthetic premixed air (Cambridge Isotope Laboratories, Andover, MA; Airco, Riverton, NJ), which contained 360 μL L^{-1} CO_2 (^{13}C 99%), 20.7% oxygen, and the balance as nitrogen, was introduced into the volatile collection apparatus by flushing the chamber at 10 L min^{-1} for 2 min and then reducing the flow to 1 L min^{-1}. The same purging procedure was followed to switch back to atmospheric air. In the initial labeling experiment, BAW feeding was initiated at 9 AM on d 1 and the labeled CO_2 air was introduced at 11 AM (d 1) for a 36-h period. Volatiles were collected at 3-h intervals continuously throughout the next 96 h. In the second set of labeling experiments BAW feeding or artificial wounding of the leaves began on d 1 at 3 PM, at which time the plants were placed in the volatile collection chambers. The $^{13}CO_2$ label was introduced on d 2 at 9 AM for a 2-h period. Plant volatiles were again collected at 3-h intervals with collections between 3 and 9 PM on d 1 and between 6 AM and 9 PM on the following 2 d.

Compounds were quantified via flame-ionization detection using the peak area of nonyl acetate (400 ng) that was added as an internal standard. To determine the amount of ^{13}C incorporated into each compound, samples were analyzed by the Finnigan MAT ITS40 (ion trap) mass spectrometer as previously described, except that the ion trap was operated in the chemical-ionization mode, with isobutane as the reagent gas. Selected mass ions were quantitated via computer software analysis. The fraction of each compound that incorporated ^{13}C was computed on a molecule basis, not on an atom basis. By summing the intensities of the $(M + 3)^+$ through $(M + n)^+$ (n = number of carbons in the molecule) ions, and comparing this with the intensity of the $(M - 1)^+$ through the $(M + 2)^+$ ion signals, we determined the contribution of the ions associated with enriched and unenriched molecules and in turn the $^{13}C/^{12}C$ ratio for each compound. Using all of the ion peaks associated with the naturally enriched volatile products ensured that enrichment levels were not overestimated. Because the molecular ion for the monoterpene linalool with its hydroxyl functional group did not appear, the $(M + 1)^+ - 18$ ion was used to calculate the ratio between the enriched and unenriched molecules. The pulse-labeling experiment was replicated three times; the resulting data were subjected to ANOVA and means were compared by an LSD test using a statistical analysis system (SAS Institute, Cary, NC). Comparisons with P values ≤0.05 were considered significantly different.

RESULTS AND DISCUSSION

Labeling Studies with $[^{13}C]CO_2$

Labeling whole cotton plants with $^{13}CO_2$, in tandem with GC-chemical ionization mass spectroscopy analysis of volatile compounds released by these plants, provided an account of the overall incorporation and a detailed picture of the distribution of the label for each compound. Thus, as illustrated in Figure 2, the ratio of labeled, indicated by relative abundances of ions above *m/z* 137 $(M + 1)^+$, to unlabeled molecules, indicated by the relative abundance of $(M + 1)^+$, of the monoterpene (*E*)-β-ocimene was greater than 9:1 after a 30-h exposure of the plants to $^{13}CO_2$. Similarly, the preponderance of molecules of (*E,E*)-α-farnesene were labeled, whereas neither β-pinene nor α-humulene incorporated a significant amount of ^{13}C during the same treatment, as indicated by the lack of appreciable abundances above the $(M + 1)^+$ ions in the spectra of the latter two compounds (Fig. 2). It is interesting that only a small fraction of the ocimene and farnesene totally incorporated the ^{13}C label, as indicated by the relative abundance of the ions at *m/z* 147 $(M + 10)^+$ and 220 $(M + 15)^+$, respectively.

Since the collection of volatiles from whole plants exposed to $^{13}CO_2$ at atmospheric concentrations did not damage the foliage, repeated sampling of the same plant during the labeling experiments was possible. In the initial experiment plants were exposed to $^{13}CO_2$ continuously for 36 h and volatiles were collected at 3-h intervals. This experiment indicated that terpene biosynthesis and/or release in response to insect feeding damage was occurring by more than one route. Several compounds incorporated substantial levels of ^{13}C within 30 h of exposure to $^{13}CO_2$ and were clearly synthesized de novo in response to insect damage. These highly labeled compounds included (*E*)-β-ocimene (92% ^{13}C enrichment), linalool (86% ^{13}C enrichment), (*E,E*)-α-farnesene (80% ^{13}C enrichment), (*E*)-β-farnesene (99% ^{13}C enrichment), and the homoterpenes (*E*)-4,8-dimethyl-1,3,7-nonatriene (93% ^{13}C enrichment) and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (54% ^{13}C enrichment).

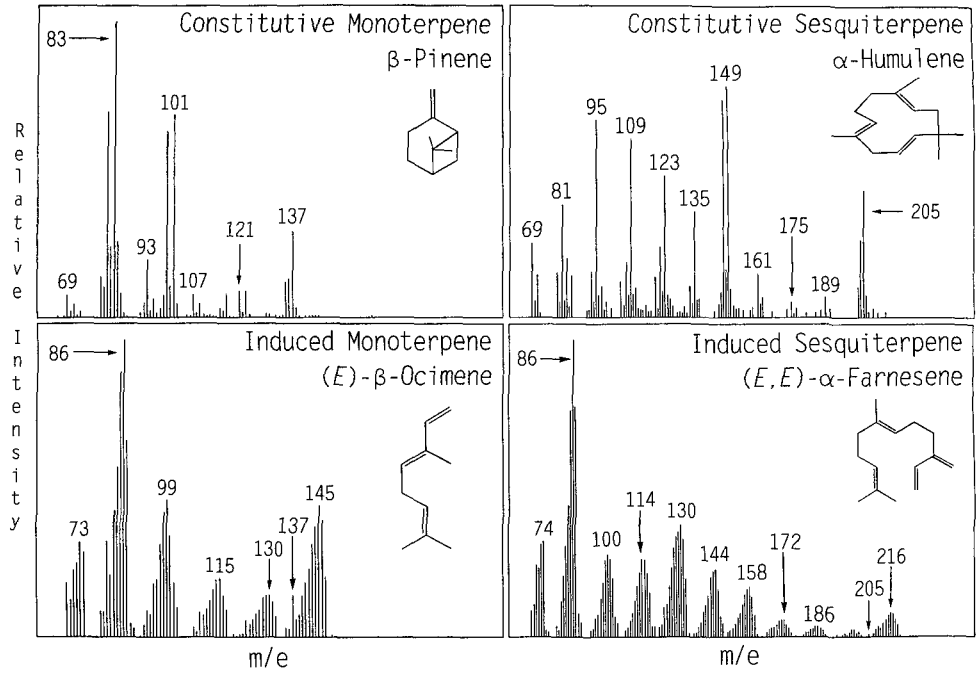

Figure 2. Chemical ionization mass spectra of representative volatile terpenes collected from cotton plants after 30 h of BAW feeding in an atmosphere consisting of 360 μL L^{-1} of >99.9% [^{13}C]CO_2 in synthetic air. The *m/z* 137 peak represents the $(M + 1)^+$ ion for the unlabeled monoterpenes and the *m/z* 205 peak represents the $(M + 1)^+$ ion for the unlabeled sesquiterpenes. m/e, *m/z*.

During the same 30-h exposure to $^{13}CO_2$ an almost equal number of volatile terpenes incorporated very low levels or nondetectable levels of ^{13}C label. Compounds with low enrichment levels included α-pinene (5% ^{13}C enrichment), β-pinene (5% ^{13}C enrichment), (*E*)-β-caryophyllene (5% ^{13}C enrichment), and α-humulene (5% ^{13}C enrichment). These volatiles were either released from storage, and thus not synthesized in response to insect wounding, or were synthesized from other precursor pools and thus did not incorporate the labeled carbon. The monoterpenes limonene and myrcene contained intermediate levels of ^{13}C label after 30 h of $^{13}CO_2$ exposure with 20 and 35% ^{13}C incorporation, respectively.

In a second experiment plants were exposed to a 2-h pulse of $^{13}CO_2$ between 9 and 11 AM on the day after feeding damage began. The highly labeled terpenes rapidly incorporated ^{13}C, as indicated by the high percentage of labeled compounds in the volatiles released during the 3-h collection period overlapping the $^{13}CO_2$ pulse (Fig. 3, far left column). Furthermore, the rapid disappearance of the carbon label from terpenes collected during the subsequent 3-h periods on that day indicated a high rate of turnover of the highly labeled compounds. Greater than one-half of the ^{13}C was displaced from the labeled terpenes within the first 3-h collection period after $^{13}CO_2$ was removed. The high synthesis rate was in agreement with a study of volatiles released from Norway spruce (*Picea abies* [L.]), in which added $^{13}CO_2$ was incorporated into monoterpenes emitted from the tree within 4 h of ^{13}C labeling (Schürmann et al., 1993). For spruce, the monoterpenes α- and β-pinene were released at high levels, even in the absence of insect damage, and both compounds emitted after $^{13}CO_2$ exposure were enriched with the isotope label. Analysis of endogenous terpenes in the needles, including α- and β-pinene, indicated that even after a 24-h exposure to $^{13}CO_2$-labeled air there was little or no difference in the level of incorporation of heavy carbon compared with those plants exposed to natural-abundance $^{12}CO_2$ air conditions. This suggests that the turnover rate of terpenes that are emitted is very different from the turnover rate of those terpenes that are stored, at least for an unwounded plant, results that are in full agreement with those obtained in the present study.

The highly labeled cotton terpenes are the same isoprenoid products recently found to be released from undamaged leaves distal to the site of damage (Röse et al., 1996). The foliage toward the top of the plant, which was distant from the leaves on which caterpillars were allowed to feed, also had an increase in the accumulation of volatiles, which we report here to incorporate only low levels of ^{13}C label. However, these products were not released into the atmosphere unless the leaves were physically damaged. The absence or low level of incorporation of the ^{13}C label during and after the 2-h $^{13}CO_2$ pulse for the unlabeled terpenes (Fig. 4, far left column) indicates a slower turnover rate for these compounds relative to the highly labeled terpenes. The incorporation rate for these low or unlabeled compounds seems to more closely parallel that for monoterpenes and sesquiterpenes that accumulate in the trichomes of unwounded plants, in which the half-life for such terpenes ranges from 5 to 170 d (Mihaliak and Lincoln, 1989; Mihaliak et al., 1991). However, until the endogenous terpe-

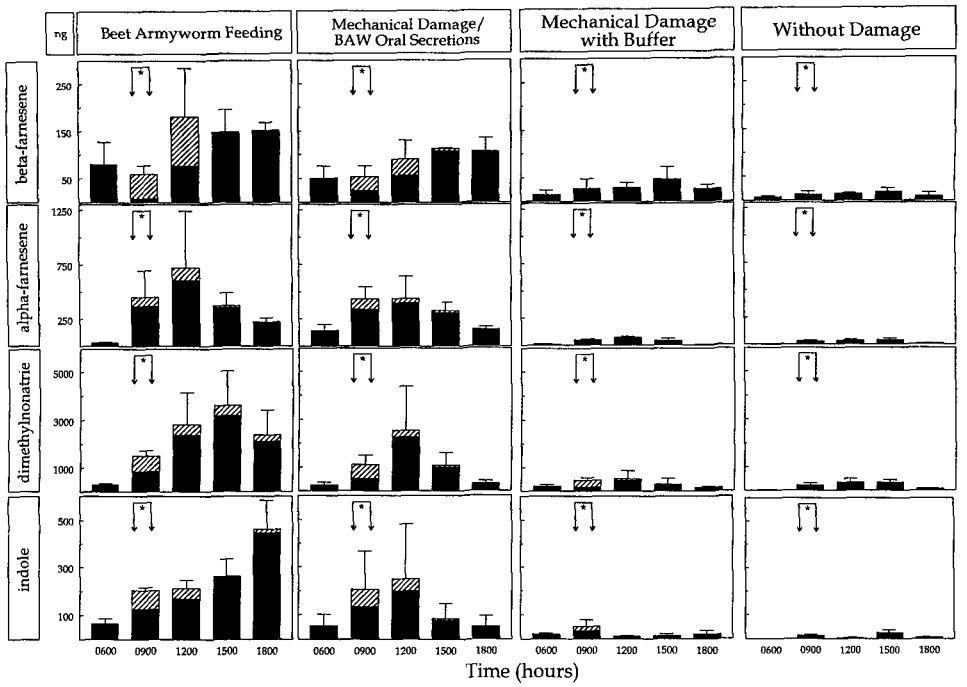

Figure 3. The release of and incorporation of ^{13}C label into highly labeled cotton volatiles with different treatments to the plant: BAW feeding, mechanical damage with BAW oral secretions, mechanical damage with buffer, and no damage. The arrows with an asterisk demarcate the period in which the plants were exposed to synthetic air highly enriched with $[^{13}C]CO_2$. The bars represent the total amounts of the volatile compound collected during a 3-h interval, which began at the indicated time; hatched portions represent the fraction of the collection that had incorporated ^{13}C label. Error bars represent SEs of the total volatile emission.

nes and those that are released are examined, an account of induction and metabolic turnover will not be complete.

The incorporation of the ^{13}C label into indole in both experiments indicated induction of a second, separate pathway, the shikimic acid / Trp pathway (Fig. 1), by insect-feeding damage. As with the induced terpenes, indole quickly incorporated the ^{13}C, and subsequently, after removal of $^{13}CO_2$ from the chamber, the label was rapidly flushed out of the pathway (Fig. 3). Unlike the highly labeled terpenes, however, indole is not released systemically in response to BAW feeding (Röse et al., 1996). Since the route of IAA biosynthesis is still a controversial issue, an increase in the rate of indole formation may be either a cause or a consequence of changes in the formation of IAA or even completely unrelated to the production of this hormone. If indole turns out to be a pivotal intermediate in the biosynthesis of the phytohormone IAA (Normanly et al., 1993; Radwanski and Last, 1995), the effect of this increase in synthesis and release of indole on synthesis and availability of IAA will be an intriguing question. It is interesting that there are several reports that low levels of herbivory can enhance plant growth (Dyer, 1980; Dyer et al., 1995).

Green leaf volatiles of the lipoxygenase pathway provided a control for de novo biosynthesis; these breakdown products of stored lipids, as expected, did not incorporate ^{13}C. The label was not detected in the lipoxygenase metabolites jasmone, (*Z*)-3-hexenal, (*E*)-2-hexenal, and the isomeric hexenyl butyrates (Loughrin et al., 1994), during either the 2- or 36-h labeling experiments. (*Z*)-3-hexenyl acetate, the only systemically released lipoxygenase volatile detected (Röse et al., 1996), contained an intermediate level of ^{13}C with 30 h of $^{13}CO_2$ exposure with 21% ^{13}C incorporation.

Insect Feeding versus Nonspecific Wound Response

Whereas the previous experiments proved that insect feeding damage induced the cotton plants to synthesize de novo some of the volatile compounds they released, the source and nature of the factor(s) responsible for both increased production and release, as well as de novo biosynthesis of the induced compounds, were not revealed. Analysis of volatiles from mechanically damaged plants, with and without application of BAW oral secretions, as well as volatiles from BAW-damaged and undamaged control plants, demonstrated that the application of the insect oral secretions increased both the production and release of ^{13}C incorporation into (*E,E*)-α-farnesene, (*E*)-β-farnesene, and indole on the 2nd d of volatile collection (Fig. 3). Furthermore, the mechanically damaged plants with caterpillar oral secretion released both farnesenes and indole in amounts similar to those found in collections from BAW-damaged plants.

The release of (*E,E*)-α-farnesene for mechanically damaged plants with BAW oral secretions exogenously applied and those plants mechanically damaged with buffer alone was significantly different on d 2 (ANOVA, LSD $P < 0.05$),

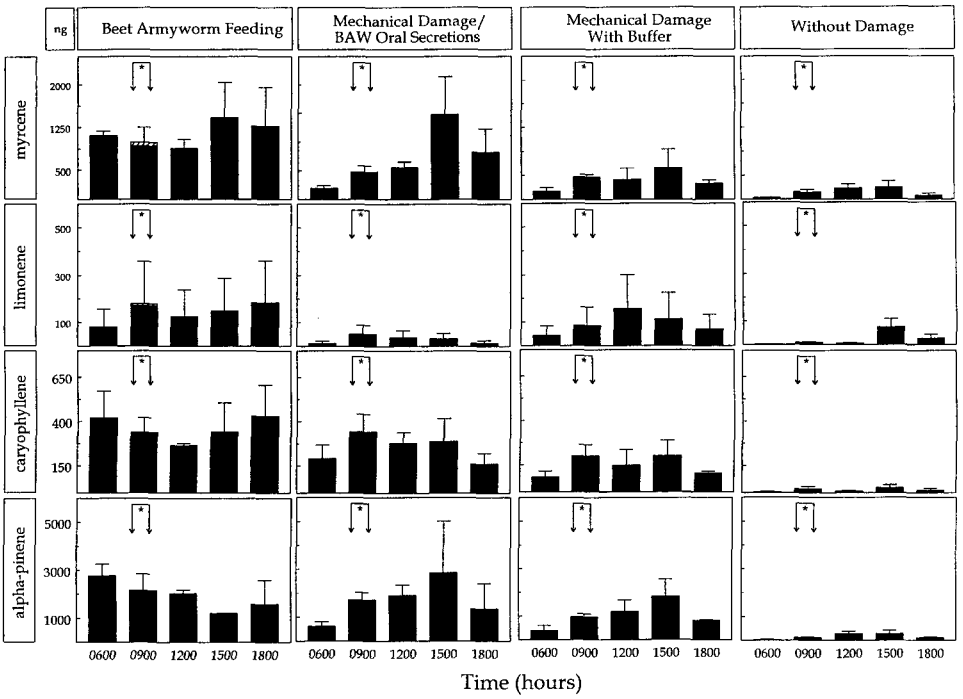

Figure 4. The profile of cotton volatiles released with little or no ^{13}C incorporation with different treatments to the plant: BAW feeding, mechanical damage with BAW oral secretions, mechanical damage with buffer, and no damage. The arrows with an asterisk demarcate the period in which the plants were exposed to synthetic air highly enriched with [^{13}C]CO_2. The bars represent the total amounts of the volatile compound collected during a 3-h interval, which began at the indicated time; hatched portions represent the fraction of the collection that had incorporated ^{13}C label. Error bars represent SES of the total volatile emission.

with almost a 10 times higher average emission rate (106 ± 31.8 ng h^{-1}) with treatment of BAW oral secretions than those treated with buffer alone (11.3 ± 5 ng h^{-1}). During the same collection period (6 AM to 6 PM on d 2), (*E*)-β-farnesene was released at an average rate of 27.7 ± 8.1 ng h^{-1} from plants with BAW regurgitant/mechanical damage versus 9.4 ± 5.1 ng h^{-1} from plants with buffer/mechanical damage (ANOVA, LSD $P < 0.05$) and indole was released at a rate of 41.2 ± 36.2 ng h^{-1} from plants with BAW regurgitant/mechanical damage versus 7.9 ± 3.8 ng h^{-1} from plants with buffer/mechanical damage (ANOVA, LSD $P < 0.05$).

Whereas mean levels of the other highly labeled terpenes that were released, including (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)-β-ocimene, and myrcene, were higher with oral secretion/mechanical damage than with buffer/mechanical damage, differences were not significant between the two treatments (ANOVA, LSD $P > 0.05$). The terpenes (some shown in Fig. 4), which incorporated little or none of the ^{13}C label, as well as limonene, linalool, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, did not show a difference in volatile release with mechanical damage either with or without BAW oral secretions exogenously applied.

To mimic the damage done by BAW feeding on the cotton leaves, an area of about 1900 mm^2 was mechanically damaged during a 48-h period, which was <5% of the total leaf area of the plant; the area of insect damage varied among plants ranging from 1750 to 3700 mm^2. Although we have yet to accurately correlate the amount of insect damage with the release of volatiles, a previous study with cotton has shown that when caterpillars are removed from the plant and there is no new damage to the foliage, the release of constitutive compounds almost immediately decreases. Thus, the release of these compounds is probably due to simple breakage of glands or cell compartments as a consequence of caterpillar feeding (Loughrin et al., 1994).

In both of the artificially damaged plants, those with exogenously applied BAW regurgitant and caterpillar-damaged plants, the percentage of incorporation of ^{13}C label was detectable for the highly labeled terpenes; in the undamaged and artificially damaged plants the percentage of incorporation for several of the compounds was below the detection limits. This may have been due to the low levels of volatiles released from the undamaged or artificially damaged plants, or this may be directly coupled with a low level of synthesis.

These findings not only demonstrate de novo biosynthesis of indole and certain terpenoids following herbivory but also demonstrate de novo synthesis from pools of newly fixed carbon. This has interesting implications for the regulation and compartmentation of isoprenoid biosynthesis. It appears that the highly enriched terpenes are tightly coupled with photosynthesis and are more responsive to the fluctuation of substrate from photosynthesis. It is interesting that preliminary analysis of volatiles from a glandless cotton variety indicate that only the highly labeled terpenes are detected with BAW feeding on the leaves (data not shown).

In this system the emission of volatiles is not activated simply by insect-applied enzymes that result in the cleavage of glycoside-bound compounds (Mattiacci et al., 1995). Several of the highly labeled volatiles were released in greater quantities as a result of caterpillar feeding or exogenously applied oral secretions from BAW plus mechanical damage than with buffer plus mechanical damage (Fig. 3). In contrast, for the unlabeled or modestly labeled products, the level of volatiles released seemed to depend directly on the amount of damage caused by either mechanical injury or by BAW feeding. The undamaged plants consistently released relatively low levels of volatiles (Figs. 3 and 4). The statistically significant difference in the release of some of the highly enriched compounds with and without the BAW regurgitant provides strong evidence that induction of synthesis of volatiles is, at least in part, triggered by an elicitor from the oral secretions of the feeding caterpillar.

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